RESEARCH ARTICLE

Synthesis and Characterization of Pure Nanosilver, Pineapple and Fig Capped Nanosilver with Potential as Smart Biomaterials for In-Vitro Studies

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ABSTRACT: Smart biomaterials materialize biomaterials as enzymes, polymers, materials and that are widely used in drug delivery and in biomedical applications. Enzymes bromelain and ficin are form of smart biomaterials present only in pineapple and fig. In the present study, pure nanosilver, pineapple and fig capped nanosilver are synthesized using sol-gel method. The surface morphology, elemental composition, functional groups and crystallinity of the samples were characterized. The antibacterial effect of nanosilver samples against two pathogenic bacteria was tested. The antioxidant potential of the samples was investigated using DPPH free radical scavenging activity. The aim of this study is to implement smart biomaterials onto nanomaterials and to utilize it for biomedical applications.

KEYWORDS: Nanosilver, Bromelain, Ficin, *M.luteus*, *P.aeruginosa* and DPPH assay

https://doi.org/10.29294/IJASE.7.3.2021.1854-1860 **© 2020 Mahendrapublications.com, All rights reserved**

INTRODUCTION

Nanotechnology is a fascinating word, in the world of science. The unique property is that the individual character of each atom, electron, photon or small objects is known. It is the toolbox that equips the nanometre sized building blocks for the origination of nanomaterials. Nanotechnology ascertains a wide range of applications almost in all the fields, being nanoscale materials and devices for effective utilization[1].

In the field of medicine and drug delivery, Biomaterials are receiving attraction from researchers, the substance engineered to interact with biological system for medical purpose. Notably, the function of biomaterials is to repair or replace a tissue in our body [2],if it lacks its function, which is leading to be employed in diagnostic techniques and therapeutic treatment. Smart Biomaterials are an emerging class of biomaterials, holding the capability to respond to the very small changes in the external stimuli, resulting in large physiological changes. These biomaterials are stimuli responsive, hence, known to be smart biomaterials. In modern medicine, they are implemented in the form of enzymes, polymers and materials. The significant property is its self-healing property [3]. Therefore, smart biomaterials are promising to be utilized in medical applications.

Silver has long been recognized as having inhibitory effect towards bacterial strains and microorganisms commonly present in medical and industrial processes [4]. Silver known to be the metal of antiquity, traditional, precious and lustrous white metal, when produced as nanomaterial exhibits aberrant properties. Nanosilver withholds a larger surface area to volume ratio and explores distinct physical, chemical and biological properties. The main advantage is that even the smaller quantities of nanosilver contains a large number of silver ions and are finely monodispersed. The salient feature of nanosilver is that it is a powerful antibacterial agent, which is immense toxic to microorganisms including bacteria and germs[5,6], is also an antiseptic agent and has anti-oxidant properties [6]. Nanosilver have a wide area of interest in various fields such as breathing tubes in medical devices, wound dressing [7], amalgams in dentistry [7,8], antibacterial socks in textile fabrics [7] and water treatment plants [9].

Nanoparticles can be synthesized by various physical and chemical methods such as laser ablation, spray pyrolysis, chemical or physical vapour deposition [10], sol-gel, mechano-chemical process and aerosol synthesis. In particular, sol-gel technique is a top-down approach to fabricate nanoparticles. The sol-gel process is a wet chemical technique [11], resulting in pure and homogeneous nanoparticles. The major advantage of sol-gel method is that it yields a large quantity of final by-product over to green synthesis method. Furthermore, on adding plant or fruit extract as reducing or capping agent for the synthesis of nanoparticles, could be advantageous [12,13].

Pineapple, scientifically called *Ananas comosus*, belongs to the family Bromeliaceae, also known as bromelain, Debridase and exquisite fruit. It is a tropical fruit, available all over the country almost in all seasons. The nutrient contents of *A.comosus* are vitamins C, B1,B5, and B6, beta-carotene, fibres and minerals. Bromelain is a proteolytic or protease

**Corresponding Author:poornibhava@gmail.com*
Received: 15.12.2020 **Accepted:** 21.01.2021 **Published on**: 22.02.2021

enzyme found only in *A.comosus*, which is present in the extract acquired from the fresh fruit or from the stem. The stability of the enzyme lasts for at least one week at room temperature. Bromelain and other nutrients in the extract make pineapple a very good source of anti-inflammatory and anti-oxidant in nature [4].

Fig, known by its scientific name, *Ficus carica*, belongs to the mulberry family, moraceae, is a soft and sweet fruit [12]. The main significance of *F.carica*, is its physical strength. The ability of fig trees to withstand in moist rainy areas and in addition to that, the endurance and exposure to water reveals the mystery and magic of Almighty Athi Varathar, is made up of Indian fig tree wood, called Athi Wood, has been survived for too many long years and still continues to survive. The nutrients contents being carbohydrates, proteins, vitamins C, E, K and types of vitamin B, minerals such as calcium is a rich source, phosphorus and potassium. Ficin is the proteolytic enzyme present especially in *F.carica*. The enzyme ficin and nutrients makes fig an abundant source of anti-oxidant.

In the present study, we report the synthesis of pure nanosilver, pineapple and fig capped nanosilver, using pineapple and fig extracts as capping agent [13,14].

MATERIALS AND METHODS

Materials

The fundamental reactants used to prepare nanosilver are, Silver nitrate, and the metal precursor. Hydrazine hydrate and sodium hydroxide are used as reducing agents. Acetic acid is used as stabilizing agent. Ammonium hydroxide solution, acetone and de-ionized water were also used to carry out this experimental work. Prior to this, the fresh, fleshy, pineapple and fig fruits are collected from organic store.

Methods

Synthesis of pure nanosilver

For the synthesis of pure nanosilver, 0.2 M of silver nitrate, 1.6 M of acetic acid and 1.6M of sodium hydroxide were added in a 250 ml borosilicate glass beaker and stirred continuously. The pH was maintained 7 by adding a drop of ammonium hydroxide solution. After an hour, 0.4 M of hydrazine hydrate was added drop-wise to the above solution. On addition, the solution suddenly turns black indicating the reduction of Ag ions and then allowed to stir continuously. After three hours, a transparent solution with shining nanosilver is obtained(11,15). The resultant gel was filtered using Whatman filter paper No.1, dried and ground well as fine nanoparticles.

Synthesis of pineapple capped nanosilver

Preparation of pineapple extract, 20 g of fresh, fleshy pulps of the fruit is collected and the basic cleaning process is made. The chopped fruit is grinded well in a blender. Then, the extract is filtered using Whatman filter paper No.1. This pineapple extract was used as capping agent.

For the synthesis of pineapple capped nanosilver, similar process was carried out as of the synthesis of pure nanosilver. After attaining the transparent solution with shining nanosilver, 10 ml of the prepared pineapple extract was added drop-wise to the above solution as a capping agent and is allowed to stir. After an hour, the resultant gel was filtered, dried and ground well as fine nanoparticles.

Synthesis of fig capped nanosilver

Preparation of fig extract, 20 g of fresh, fleshy pulps of the fruit is collected and the basic cleaning process is made. The chopped fruit is grinded well in a blender. Then, the extract is filtered using Whatman filter paper No.1. This fig extract was used as capping agent. The fig capped nanosilver was synthesized alike the preparation of pineapple capped nanosilver.

The characterization of nanoparticles examines the physical, chemical and biological behaviour through various microscopic and spectroscopic techniques. The as-synthesized nanoparticles are characterized by XRD, SEM – EDAX, FTIR studies and *in-vitro* studies such as anti-bacterial and anti-oxidant activity.

RESULTS AND DISCUSSION

XRD Analysis

The crystalline nature of nanoparticles was confirmed by x-ray crystallography. The x-ray diffraction spectra of all the samples are shown in Figure 1, that has the characteristic peaks at around 38°, 44°, 64° and 77° corresponds to the lattice planes (111), (200), (220) and (311) respectively.

Table 1. The results of XRD Data

Parameters	Samples		
	Pure Nano silver	Pine apple capped Nanosilver	Fig capped Nanosilver
Crystal Size(nm)	28.10	24.87	17.56
Lattice constant(Å)	4.0967	4.0849	4.0885
Volume of a Unit Cell(Å)^3	68.75	68.16	68.34

The diffraction peak is plotted against the Bragg reflections at 2θ to the corresponding lattice planar intensities. As consistent with the previous studies (9,11,12,14), the XRD spectra and the parameters of all the samples are in good agreement with the JCPDS card number (04-0783) and the unit cell of face centered cubic structure.

From the obtained lattice planes, it is confirmed that all the samples attain face centered cubic structure, the lattice constant 'a' is calculated for the more intense peak to its respective inter-planar d spacing and the corresponding unit cell volume is found. As shown in Table 1, the average crystal size, lattice constant and unit cell volume for each sample are tabulated.

SEM Analysis

The surface morphology of the three samples is noticed by the SEM micrographs in figure 2. The surface of pure nanosilver under SEM analysis appears to be sub-rounded granular in shape [11]. The particles are crystalline and attain a larger surface area. The pineapple capped nanosilver particles appear to be mostly spherical, while, the fig capped nanoparticles are spherical like shaped in appearance. In an earlier study [14,16] also obtained formation of spherical shaped silver nanoparticles.

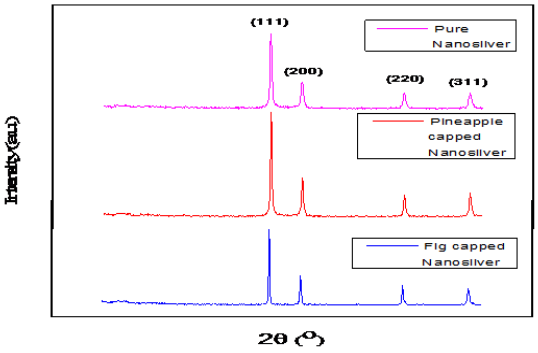

Figure 1. XRD Spectra of three samples

The surface morphology is well known from the SEM analysis, which reveals the purpose of adding capping agent. In Pure nanosilver without capping agent, the particles are nucleated and have led to agglomeration. So, that the particles are large as compared to that of the other two samples. The addition of pineapple extract and fig extract to nanosilver, as a capping agent, had played a significant role in attaining the nanosize and has also prevented agglomeration in pineapple and fig capped nanosilver specimens.

EDAX Analysis

The EDAX spectrum identified the elements present in each sample and recorded the composition of each element. The spectrum attaining the highest peak in figure 3, (a) confirms the presence of the element silver and some amount of oxygen in pure nanosilver. Figure 3 (b) represents the spectrum of pineapple capped nanosilver. The apical peak approves the presence of silver element and the small peaks represent the minerals present in pineapple extract such as manganese, aluminum and magnesium. The existence of the element Ag from the topmost peak and diminutive peaks affirms the presence of various minerals such as magnesium, sodium and calcium in fig extract and small percentage of oxygen are shown in figure 3.(c) indicating fig capped nanosilver. Other elemental signals are recorded possibly due to elements from enzymes or proteins present within the capped samples [17]. Pure nanosilver and fig capped nanosilver are oxidized to some extent whereas pineapple capped nanosilver has not oxidized. Table 2 lists the weight percentage of each element and minerals present in the three samples.

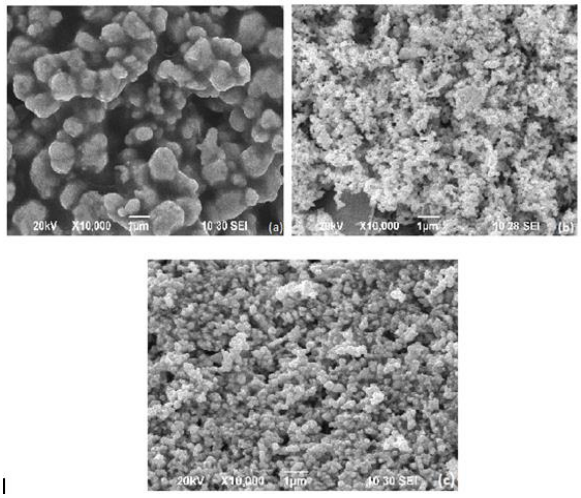

Figure 2. SEM micrographs of three samples

FT-IR Analysis

The FT-IR spectra and the characterized functional groups of the samples are illustrated in Figure 4. In all the spectra, the similar bands are present as noted in the previous studies(9,18–21). The bands detected around 3400 cm^{-1} – 3500 cm^{-1} and 1000 cm^{1} - 1300 cm^{-1} corresponds to O-H stretching vibrational modes of phenolic group. The bands associated to the characteristic stretching modes of C-H methylene group of proteins were observed at 2829 cm^{-1} – 2926 cm^{-1} respectively. The bands at around 1630 cm^{-1} – 1640 cm^{-1} correspond to C-C stretching vibrations indicating the presence of proteins. The typical bands at 1514 cm^{-1} – 1562 cm^{-1} were attributed to the amide II linkage of proteins.

In-vitro Antibacterial Activity

The i*n-vitro* antibacterial assay of the as-prepared samples was assessed by disc diffusion agar

method(22), that reveals the potential of the samples against two microorganisms.

Micrococcus luteus is a gram-positive bacterium, which induces skin borne infections, bacteraemia, meningitis, arthritis and is also fatal to leukemia patients causing from pulmonary haemorrhages.

Pseudomonas aeruginosa is a gram-negative bacterium, which is responsible for causing high risk factors such as lowering the concentration of neutrophils (WBC) in blood, chronic pneumonia, skin and soft tissue and also infects burns, affects wound infections.

The antibacterial activity of the three samples labelled as A – Pure nanosilver, P – Pineapple capped nanosilver and F – Fig capped nanosilver against the mentioned two organisms are represented in Figure 5. The diameters of each zone of inhibition are measured and the data are tabulated in Table 3.

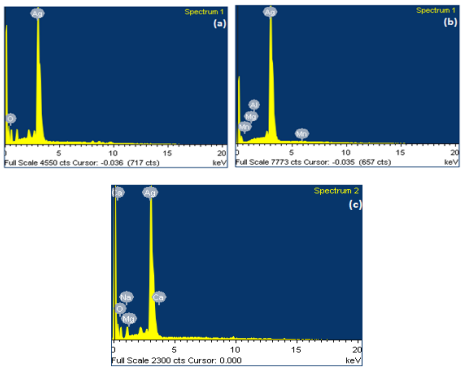

Figure 3. EDAX Analysis of three samples

Table 2. The results of EDAX Analysis

Elements (at. %)	Samples		
	Pure Nanosilver	Pineapple Capped Nanosilver	Fig capped Nanosilver
Ag	82.27	99.22	79.64
O	17.73	-	15.14
Mn	-	0.10	-
Mg	-	0.32	0.03
Al	-	0.36	-
Na	-	-	5.16
Ca	-	-	0.03

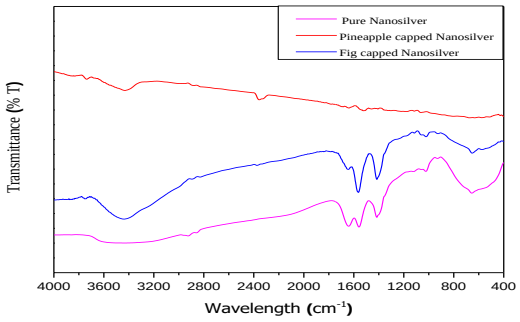

Figure 4. FT-IR Spectra of three samples

Pure nanosilver has higher inhibitory level, since it contains a large number of silver ions and also attains a larger surface area. Whereas, the inhibitory level of the capped samples is remarkably low than the inhibitory level of pure sample, due to the contents present in the capping agent. As the result of antibacterial activity, pure nanosilver has higher inhibitory level than the other two samples and is suggested to be a promising antibacterial agent.

In-vitro Antioxidant Activity

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Basically, free radical is nothing but an uncharged molecule having an unpaired valence electron. Oxidation is the process of loss of electrons, which produces free radicals; these free radicals may damage the cells of living organisms. DPPH (Diphenyl-2-picrylhydrazyl) the stable free radical scavengers, called DPPH assay is used to evaluate the radical scavenging ability of the extracts. Reduction is the process of gaining of electrons. The DPPH assay is based on the reduction of DPPH. When antioxidants (hydrogen ion donors) react with DPPH (free radicals) it becomes paired off.

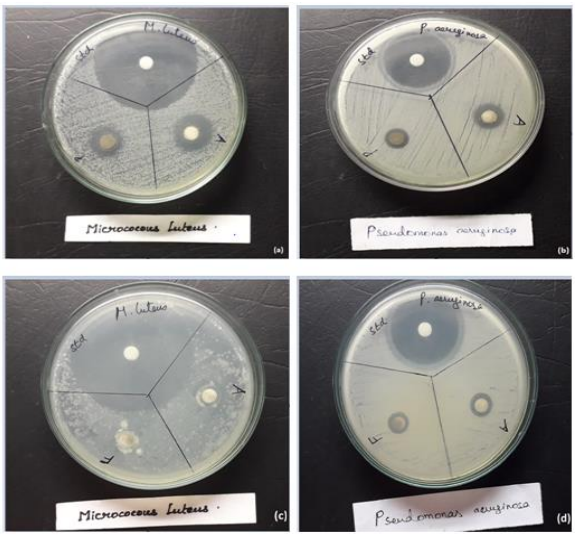

Figure 5. Images of Antibacterial activity

The hydrogen donating ability of extracts was examined. The extract solution was made by dissolving the sample in distilled water. The DPPH solution in ethanol was made and 1.0 ml of the solution is added to 3.0 ml of extract solution in water at different concentrations. After 30 minutes, the DPPH gives maximum absorption at 517 nm. The DPPH with ethanol is taken as negative control. Ascorbic Acid was considered as positive control. The DPPH solution is purple color; on reacting with antioxidants, it is reduced to DPPH-H, which is yellow in colour indicating that absorbance decreases. More the decolourization more is the reducing ability[23,24]. The antioxidant activity of the extract was expressed as inhibitory concentration IC_{50}. Lower the IC_{50} value, Higher is the antioxidant activity.

The capability to scavenge the DPPH radical was calculated using the equation,

% Scavenging/Inhibition = [Absorbance of control – Absorbance of test sample / Absorbance of control] × 100

The absorbance and corresponding percentage inhibition for each standard ascorbic acid and samples are tabulated in Table 4. Ascorbic acid is nothing but vitamin C that has minimal inhibitory concentration to free radicals which means higher antioxidant ability. The nearer values of pineapple and fig capped nanosilver to the standard ascorbic acid confirms the presence of vitamins, proteins, minerals and smart biomaterial enzymes bromelain and fic in [13,14], that substantiates the antioxidant properties of pineapple and fig capped nanosilver [20] than pure nanosilver. Therefore, pineapple and fig capped nanosilver can be implied to be effective antioxidants.

CONCLUSION

The analysis of XRD, SEM – EDAX and FT-IR showed that particles of all samples are of nanosized, homogeneous and explain the effect of capping agent. The antibacterial efficiency of all samples affirms that pure nanosilver attain quite promising antibacterial properties which may be valuable in treatment of various infections and diseases induced by the two organisms *M.luteus* and *P.aeruginosa*than the capped samples. *In-vitro* Antioxidant activity of all samples confess that pineapple and fig capped nanosilver are more effective antioxidants than pure nanosilver. Hence, these beneficial antioxidants may be used to protect our living cells which might fight against the diseases induced by the free radicals. This study is a primary measure towards the implementation of smart biomaterials onto nanomaterials. The future study will be qualitative and quantitative analysis of smart biomaterials and utilize them for biomedical applications.

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Table 3. The results of Antibacterial Activity

S.No.	Organisms	Zone of Inhibition (mm)			
		Pure Nanosilver (100 µg / disc)	Pineapple capped Nanosilver (100 µg / disc)	Pure Nanosilver (100 µg / disc)	Fig capped Nanosilver (100 µg / disc)
1.	*Micrococcus luteus*	15	11	11	10
2.	*Pseudomonas aeruginosa*	21	16	12	11

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Table 4. The results of Antioxidant activity

Conc.	Ascorbic Acid		Pure Nanosilver		Pineapple capped Nanosilver		Ascorbic Acid		Fig capped Nanosilver	
	Abs.	% Inhibit	Abs.	% Inhibit	Abs.	% Inhibit	Abs.	% Inhibit	Abs.	% Inhibit
10	0.188	24	0.206	16	0.194	21	0.542	20	0.586	14
20	0.164	33	0.182	26	0.184	24	0.486	23	0.502	27
40	0.142	42	0.164	33	0.168	32	0.412	34	0.466	32
60	0.126	49	0.147	40	0.152	38	0.362	41	0.388	43
80	0.106	56	0.126	49	0.144	42	0.286	58	0.298	56
100	0.086	65	0.112	52	0.128	48	0.194	72	0.204	70
IC_{50} Values	IC_{50} = 27.27(µg/ml)		IC_{50} = 34.05 (µg/ml)		IC_{50} = 31.28 (µg/ml)		IC_{50} = 66.70 (µg/ml)		IC_{50} = 68.38 (µg/ml)	

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